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Alkanoylsucroses in nasal delivery of low molecular weight heparins: in-vivo absorption and reversibility studies in rats

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Abstract

The efficacy of alkanoylsucroses in enhancing nasal absorption of low molecular weight heparin (LMWH) and the time span of action of these agents on the nasal membrane has been investigated. In this regard, LMWH formulated with alkanoylsucroses was administered nasally to anaesthetized male Sprague-Dawley rats and the absorption of LMWH was determined by measuring plasma anti-factor Xa activity. The duration of action of these agents at the site of administration was investigated by an in-vivo reversibility study. The potency and efficacy of dodecanoylsucrose was compared with that of sodium glycocholate. Alkanoylsucroses used in this study include dodecanoylsucrose, decanoylsucrose and octanoylsucrose. These agents enhance nasal absorption of enoxaparin in a dose-dependent and chain-length-dependent manner. Of the agents tested, dodecanoylsucrose was found to be the most potent in enhancing nasal absorption of LMWH. The bioavailability of enoxaparin formulated with alkanoylsucroses was increased by several folds compared with enoxaparin formulated in saline. The reversibility study with dodecanoylsucrose showed that the effect of alkanoylsucroses faded away with time and the duration of action of this agent at the site of administration was 120–140 min. Dodecanoylsucrose was found to be twice as potent as sodium glycocholate. Overall, the nasal absorption of LMWH was effectively enhanced by co-administration of alkanoylsucroses and the effect of alkanoylsucroses on nasal epithelium was found to be reversible. The potency of these agents depends on their hydrophobic chain lengths.

Introduction

Low molecular weight heparins (LMWHs) are fragments of natural heparin produced by enzymatic or chemical depolymerization of unfractionated heparins (UFH) (Thanou et al 2001). In recent years, LMWH has been used as an alternative to unfractionated heparin in the treatment of deep vein thrombosis and pulmonary embolism. LMWH therapy is preferred to conventional heparin therapy, because LMWHs can be delivered subcutaneously with a bioavailability greater than 90% and have a much longer half-life. In addition, LMWHs do not bind at secondary locations, as does UFH, and thus do not cause many of the untoward effects associated with UFH. However, one of the major disadvantages of LMWH therapy is that it needs to be administered by the subcutaneous or intravenous route. Concerns have been raised about patient compliance, longer hospital stay and requirement of skilled health professionals for therapeutic drug monitoring and administration. These concerns have prompted interest in developing alternative drug delivery systems including oral, transdermal and nasal delivery of LMWH (Mitragotri & Kost 2001; Thanou et al 2001; Arnold et al 2002).

In recent years, the nasal route has emerged as a route of choice for the systemic administration of drugs that are ineffective orally and must be administered by injection. Because of rich vasculature, avoidance of first-pass effect and ease of self administration, the nasal route has been proposed as a convenient and safe route of administration for non-invasive delivery of peptide and protein drugs (Ishikawa et al 2002). However, nasal drug delivery has been plagued with several disadvantages, including toxicity of absorption promoters to the nasal membrane and impermeability of the nasal mucosa to

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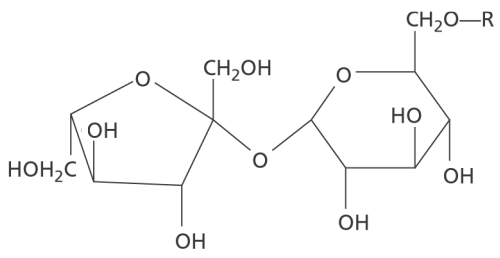
prospective drug candidates (Behl et al 1998; Ugwoke et al 2001). To overcome the nasal muscosal barrier, drugs intended for nasal delivery need to be co-administered with absorption promoters (O'Hagan & Illum 1990; Lee et al 1991; Donovan & Huang 1998). Bile salts and various nonionic surfactants have been widely used as absorption promoters for nasal delivery of peptide drugs. For instance, use of sodium glycocholate in nasal formulation is well documented. This agent has been extensively studied as an absorption promoter for nasally administered peptide drugs such as insulin, calcitonin, and recombinant growth hormone (Pontiroli et al 1987, 1989; Aungst et al 1988). Unfortunately, the use of sodium glycocholate has not been pursued towards the development of a safe and viable nasal formulation of any peptide drugs, perhaps because of the toxic effects of this agent at the site of administration (Hirai et al 1981; Lee et al 1991; Merkus et al 1993).

Recently, alkylglycosides have been introduced as potential absorption promoters for nasally administered drugs such as insulin and calcitonin (Pillion et al 1994; Ahsan et al 2001). Chemically, alkylglycosides are nonionic surfactants that contain a sugar moiety attached to a hydrophobic chain of variable length by ether or ester linkage. The sugar moiety in alkylglycosides may include maltose, sucrose or glucose (Figure 1). In a recent study, it has been shown that the efficacy of alkylglycosides, containing either maltose or sucrose as sugar moiety, in enhancing nasal absorption of insulin increases with an increase in the hydrophobic chain length (Pillion et al 2002). In another study (Arnold et al 2002), it was reported that tetradecylmaltoside, an alkylglycoside containing a maltose ring attached to a 14-carbon alkyl chain, enhances nasal absorption of LMWHs, enoxaparin and dalteparin. However, there is no data as to the use of alkanoylsucroses, alkylglycosides containing a sucrose ring as the sugar moiety, as absorption promoters for nasally administered macromolecules other than peptide drugs (Pillion et al 2002). Based on the effects of alkanoylsucroses on nasal peptide absorption, one cannot predict the effect of these agents on nasal absorption of LMHWs, a different family of negatively charged molecules.



R = $C_{12}H_{23}O$ n-Dodecanoylsucrose

R = $C_{10}H_{19}O$ n-Decanoylsucrose

R = $C_8H_{15}O$ n-Octanoylsucrose

Figure 1 Chemical structure of alkanoylsucroses.

Furthermore, it is not known if the potency, efficacy and duration of action of these agents are comparable with those of widely used nasal absorption promoters such as sodium glycocholate.

The purpose of this study is to test the hypothesis that alkanoylsucroses enhance nasal absorption of enoxaparin, a model drug for LMWHs, and to determine whether these agents enhance nasal absorption in a hydrophobic-chain-length- and time-dependent manner. In this regard, enoxaparin was formulated with three alkanoylsucroses, namely, dodecanoylsucrose, decanoylsucrose and octanoylsucrose containing a 12-, 10- and 8-carbon alkyl chain, respectively. The efficacy and duration of these agents were compared with those of a known nasal absorption promoter, sodium glycocholate.

Materials and Methods

Materials

Enoxaparin injection (Lovenox) was obtained from Aventis Pharmaceuticals Products Inc (Bridgewater, NJ) as a sterile solution containing 30 mg of enoxaparin sodium (3000 U of anti-Factor Xa activity) per 0.3 mL. Dodecanoylsucrose, decanoylsucrose and octanoylsucrose were purchased from Calbiochem-Novabiochem Corporation (La Jolla, LA). Sodium glycocholate was obtained from Sigma Chemicals (St Louis, MO).

Preparation of formulations

Stock solutions of the three alkanoylsucroses (dodecanoyl-, decanoyl- and octanoylsucrose) were prepared by dissolving the reagents in normal saline at 1% and stored at 4 °C for 30 days or less. Stock solutions older than 30 days were not used in the experiments. It had previously been determined (data not shown) that there were no differences in the drug absorption when formulations containing reagents stored for 30 days were used, compared with formulations that contained freshly prepared alkanoylsucrose solutions. Formulations for nasal absorption studies were prepared by mixing enoxaparin with saline or appropriate concentrations of different alkanoylsucroses. The concentrations of alkanoylsucroses used in the formulations were 0.125, 0.25 and 0.5%, which were selected based on earlier studies on alkylmaltosides and alkanoylsucroses as nasal absorption enhancers (Pillion et al 1994, 1998, 2002; Ahsan et al 2001, 2003a). The strength of the final formulation was such that each 20 μL of the solution contained an amount of enoxaparin sodium (1 mg) equivalent to 100 U of anti-factor Xa activity. Similarly, for subcutaneous and intravenous absorption studies, formulations were made by diluting enoxaparin with an appropriate amount of saline. Formulations for subcutaneous administration were prepared to contain 100 U of anti-factor Xa activity in each 100 μL of solution and formulations for intravenous administration contained 100 U of anti-factor Xa activity in each 200 μL of solution. The concentration of sodium glycocholate used in this study was 1% and the preparation of nasal enoxaparin

formulation containing sodium glycocholate was the same as the alkanoylsucrose formulations described above. The concentration of sodium glycocholate (1%) was selected based on earlier studies on in-vivo nasal drug delivery (Marttin et al 1995).

Nasal absorption studies in rats

Male Sprague-Dawley rats (Charles River laboratories, Charlotte, NC), 250–350 g, were used for the in-vivo absorption experiments (3–5 rats in each group). Before the experiment, the rats were anaesthetized by an intramuscular injection of an anaesthetic cocktail containing xylazine (30 mg kg^{-1}), ketamine (30 mg kg^{-1}) and acepromazine (10 mg kg^{-1}). Anaesthesia was maintained with additional intramuscular injections of anaesthetic solution as needed throughout the experiments. Samples (about 20 μL) of the formulations (330 U kg^{-1}) were instilled to the left nare of the rat, using a pipetter with a disposable plastic tip. Only one nostril received the formulation to avoid blockage of the airway. Rats were maintained in the supine position for at least 5 min to ensure adequate contact time between the epithelium and the formulation. For bioavailability studies, formulations were administered either subcutaneously or intravenously. For subcutaneous administration, formulations were administered as a single 100-μL injection under the back skin and for intravenous administration, a 200-μL bolus injection was administered via the tail vein. After nasal and subcutaneous administration, blood samples (about 300 μL) were collected from the tip of the rat tail at 0, 30, 60, 120, 240 and 360 min in citrated microcentrifuge tubes and placed on ice; after intravenous administration, blood was collected from the orbital vein. Subsequently, plasma was separated by centrifugation (1600 *g*, 5 min) and was stored at −20 °C until further analysis. Enoxaparin absorption was determined by measuring plasma anti-factor Xa levels using a colorimetric assay kit (Chromogenix Coatest Heparin Kit; Diapharma Group Inc., West Chester, OH).

Reversibility studies

The in-vivo reversibility study was performed as reported earlier (Vermehren et al 1996; Bagger et al 2001). For these experiments, formulations were administered in two phases. In the first phase, 20 μL of formulation containing 0.5% dodecanoylsucrose or 1% sodium glycocholate, without enoxaparin, was administered to the left nare at time zero. In the second phase, formulations containing only enoxaparin, equivalent to 100 U of anti-factor Xa in each 20 μL, were administered to the same nostril immediately afterwards (time zero) or at 60 or 120 min after the first phase of administration. In these sets of experiments, blood samples were collected at 0, 5, 15, 30, 45, 60, 90 and 120 min as described above.

All studies were approved by the Texas Tech University Health Sciences Center (TTUHSC) Animal Care and Use Committee (Protocol no. AM 02004-02) and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Pharmacokinetic analysis

Standard non-compartmental analysis (Kinetica, Version 4.0; Innaphase Corp., Philadelphia, PA) was performed for enoxaparin absorption profiles. The area under the plasma concentration versus time curve ($AUC_{0\rightarrow360}$ or $AUC_{0\rightarrow120}$) was calculated by the trapezoidal method. Absolute and relative bioavailability ($F_{absolute}$ and $F_{relative}$) was estimated by comparing $AUC_{0\rightarrow360}$ for nasally administered enoxaparin with that of intravenously and subcutaneously administered enoxaparin, respectively.

Statistical analysis

Pharmacokinetic parameters of different formulations were compared by analysis of variance. When the differences in the means were significant, post-hoc pair-wise comparisons were conducted using Newman–Keuls multiple comparison (GraphPad Prism, version 3.03; GraphPad Software, San Diego, CA). Differences in *P*-values less than 0.05 were considered statistically significant.

Results and Discussion

Nasal absorption and bioavailability studies

To investigate the efficacy of alkanoylsucroses as nasal absorption enhancers of LMWHs, enoxaparin was formulated with three alkanoylsucroses of varying hydrophobic chain lengths and administered to anaesthetized rats. Enoxaparin formulated in saline without alkanoylsucroses failed to produce a plasma anti-factor Xa level required for an antithrombotic effect in male Sprague-Dawley rats (> 0.20 U mL^{-1}, Bianchini et al 1995). However, administration of a nasal formulation containing enoxaparin plus different alkanoylsucroses increased the plasma anti-factor Xa activity to different extents (Figure 2). When enoxaparin formulated with 0.125% octanoylsucrose was administered as a nose drop, no appreciable increase in plasma anti-factor Xa level was observed compared with enoxaparin formulated in saline (Figure 2A). Similarly, enoxaparin plus 0.25% octanoylsucrose formulation failed to produce a therapeutic level of anti-factor Xa. Nevertheless, formulation containing 0.5% octanoylsucrose barely achieved a therapeutic level of anti-factor Xa activity, producing a maximum effect 60 min after nasal instillation. Like formulations containing 0.125% octanoylsucrose, enoxaparin plus 0.125% decanoylsucrose was unable to produce a therapeutic level of anti-factor Xa (Figure 2B). The failure of octanoylsucrose and 0.125% decanoylsucrose to enhance nasal absorption of enoxaparin could be attributed to their critical micelle concentrations (CMCs). In fact, all concentrations of octanoylsucrose used in the study were below its CMC, 1.14% (Table 1). Since the CMC of decanoylsucrose is 0.124%, enoxaparin formulated with 0.125% decanoylsucrose was unable to cause a significant increase in nasal absorption of enoxaparin ($P > 0.05$). When the concentration of decanoylsucrose was increased to 0.25% or 0.5%,

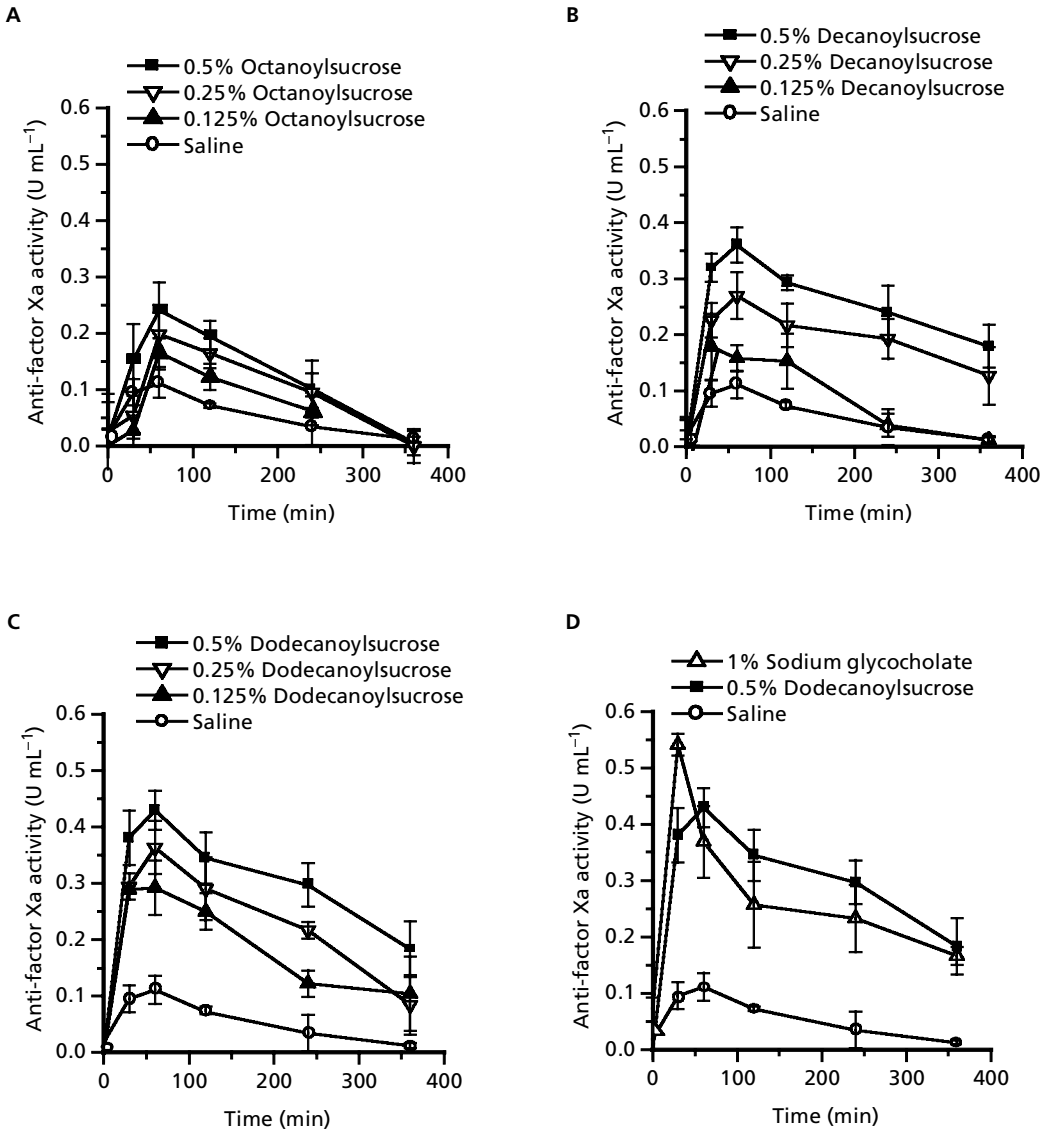


Figure 2 Changes in anti-factor Xa activity after nasal administration of enoxaparin formulated in saline or in the presence of different concentrations of octanoylsucrose (A), decanoylsucrose (B), dodecanoylsucrose (C) and sodium glycocholate and dodecanoylsucrose (D) to anaesthetized rats (enoxaparin dose, 330 U kg^{-1}). Data represent mean ± s.e.m., n = 3–5.

there was a significant and rapid increase in the absorption of enoxaparin ($P < 0.05$) (Figure 2B). Unlike octanoyl- and decanoylsucrose, all formulations containing dodecanoylsucrose produced an anti-factor Xa level that was well above the concentration required for therapeutic effect of enoxaparin (Figure 2C). Overall, the data presented in Figures 2 B and C demonstrate that decanoyl- and dodecanoylsucrose were efficacious in enhancing nasal absorption of enoxaparin and that enoxaparin absorbed via the nasal route was able to increase anti-factor Xa level. The data also suggest that there was a dose-dependent and chain-length-dependent effect of alkanoylsucrose on the nasal absorption of enoxaparin. Of the formulations tested, enoxaparin plus 0.5% dodecanoylsucrose was found to be the most potent. To compare the efficacy and potency of dodedecanoyl sucrose with that of a known nasal absorption promoter, enoxaparin was formulated with 1% sodium glycocholate and administered nasally. Like 0.5% dodecanoylsucrose, 1% sodium glycocholate produced a rapid and substantial increase in anti-factor Xa level ($P < 0.05$) (Figure 2D). The absorption profile of the formulation containing enoxaparin plus 0.5% dodecanoylsucrose was comparable with that produced by enoxaparin plus 1% sodium glycocholate, suggesting that dodecanoylsucrose is twice as potent as sodium glycocholate.

The enoxaparin C_{max} for the formulation containing 0.5% dodecanoylsucrose was higher than for any of the

Table 1 Pharmacokinetic parameters for formulations containing enoxaparin in saline or in different alkanoylsucroses at various concentration, as well as 1% sodium glycocholate, following nasal administration to rats.

Absorption enhancer	Concn	C_{max} ($U\,mL^{-1}$)	T_{max} (min)	$AUC_{0\rightarrow360}$ ($U\,min\,mL^{-1}$)	$F_{absolute}$ (%)	$F_{relative}$ (%)
None	—	0.15 ± 0.02	66 ± 15	21.3 ± 1.8	4.8 ± 0.4	5.1 ± 0.5
Octanoylsucrose	0.125%	0.16 ± 0.02	60	23.4 ± 1.1	5.2 ± 0.2	5.6 ± 0.3
(1.143%)	0.25%	0.20 ± 0.04	60	31.9 ± 4.0	7.2 ± 0.9	7.7 ± 1.0
	0.5%	$0.27 \pm 0.03^{a,b}$	80 ± 20	$42.7 \pm 5.8^{a,b}$	$9.6 \pm 1.3^{a,b}$	$10.3 \pm 1.4^{a,b}$
Decanoylsucrose	0.125%	0.23 ± 0.02	70 ± 25	29.8 ± 4.4	6.6 ± 1.0	7.2 ± 1.1
(0.124%)	0.25%	$0.28 \pm 0.03^{a,b}$	50 ± 10	$67.8 \pm 12.0^{a,b}$	$15.2 \pm 2.7^{a,b}$	$16.4 \pm 2.9^{a,b}$
	0.5%	$0.36 \pm 0.03^{a,b}$	80 ± 30	$90.0 \pm 7.6^{a,b}$	$20.2 \pm 1.7^{a,b}$	$21.7 \pm 0.8^{a,b}$
Dodecanoylsucrose	0.125%	0.32 ± 0.02^{a}	50 ± 10	63.9 ± 12.2^{a}	14.3 ± 2.7^{a}	15.4 ± 2.9^{a}
(0.015%)	0.25%	0.38 ± 0.03^{a}	60 ± 20	79.0 ± 10.7^{a}	17.7 ± 2.4^{a}	19.1 ± 2.6^{a}
	0.5%	$0.43 \pm 0.03^{a,b}$	50 ± 10	$107.8 \pm 6.7^{a,b}$	$24.2 \pm 1.5^{a,b}$	$26.0 \pm 1.6^{a,b}$
Sodium glycocholate	1%	0.54 ± 0.02^{a}	30	94.9 ± 16.3^{a}	21.3 ± 3.6^{a}	22.9 ± 3.9^{a}
Subcutaneous	—	1.19 ± 0.07	200 ± 80	414.8 ± 9.1	93.0 ± 2.1	—
Intravenous	—	3.66 ± 0.14	0	446.0 ± 22.2	100	—

The values in parentheses represent CMC (Calbiochem-Novabiochem Catalogue 2003, La Jolla, LA). Data represent mean ± s.e.m., n = 3–5; $^{a}P < 0.05$ compared with the formulation without enhancer (control); $^{b}P < 0.05$ compared with the formulation containing 0.125% alkanoylsucroses.

other formulations apart from that containing 1% sodium glycocholate (Table 1). For example, the C_{max} for the 0.5% dodecanoylsucrose formulation was approximately twice that for the formulation containing the same concentration of octanoylsucrose. As can be seen in Table 1, with an increase in the concentration of alkanoylsucrose, there was an increase in the C_{max} of the drug absorption profile. It is interesting to note that T_{max} for all alkanoylsucrose formulations was close to 60 min, while T_{max} for sodium glycocholate was 30 min. To assess the feasibility of nasal enoxaparin as an efficacious delivery system, absolute and relative bioavailability was calculated by comparing the bioavailability of nasally administered enoxaparin with that of subcutaneously and intravenously administered enoxaparin. A dose- and chain-length-dependent effect can be appreciated from the $AUC_{0\rightarrow360}$ and bioavailability data presented in Table 1 and Figure 3. When the concentration of alkanoylsucrose was increased from 0.125% to 0.5%, there was a 2- to 3-fold increase in both absolute and relative bioavailability. For example, when the concentration of decanoylsucrose was increased from 0.125% to 0.5%, relative bioavailability increased 3 fold (i.e. from 7% to 21%). Similarly, when the chain length of alkanoylsucrose was increased from 8 to 12 carbons, the absolute and relative bioavailability was increased by several fold. The absolute and relative bioavailability for 1% sodium glycocholate formulations was approximately the same as that obtained from formulations containing 0.5% dodecanoylsucrose, substantiating the fact that dodecanoylsucrose is twice as potent as sodium glycocholate in enhancing nasal absorption of enoxaparin. The relative bioavailability data suggest that nasal enoxaparin could be as effective as subcutaneous enoxaparin and that nasal delivery of LMWH could be a viable and non-invasive alternative to subcutaneous LMWH. However, caution should be exercised during interspecies scaling of nasal formulations. Significant interspecies differences were observed for nasal insulin formulated with different absorption enhancers (Merkus et al 1999). The authors argued that this variability was partly because the pre-clinical studies were conducted in anaesthetized animals, while clinical trials were performed in conscious human subjects. However, we believe that this interspecies variability was because of the difference in the type and level of enzymes present in the nose of man and rats. Interspecies differences may not pose a problem for nasal LMWH since LMWH does not undergo enzymatic degradation to the same extent as that observed in nasal insulin delivery.

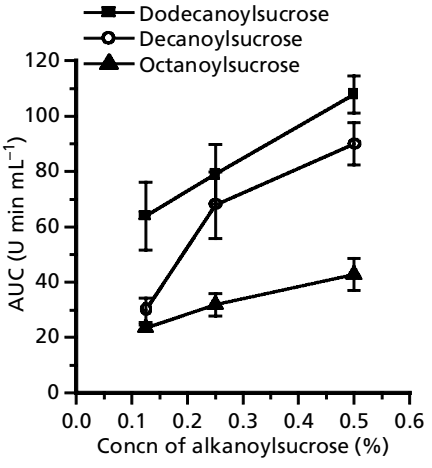


Figure 3 Changes in $AUC_{0\rightarrow360}$ with the alkanoylsucrose concentrations. Data represent mean ± s.e.m., n = 3–5.

Data presented thus far show that the efficacy of alkanoylsucroses depends largely on their concentration and hydrophobic chain length. This concentration- and chain-length-dependent effect of alkanoylsucrose can be illustrated by plotting $AUC_{0\rightarrow360}$ against concentration or hydrophobic chain length of alkanoylsucroses. As can be seen in Figure 3, the $AUC_{0\rightarrow360}$ for different formulations increases with the increase in concentrations. In fact, there was a linear, or near linear, relationship between the concentration used and the $AUC_{0\rightarrow360}$ obtained (for dodecanoylsucrose, $r = 0.98$; for decanoylsucrose, $r = 0.89$; for octylsucrose, $r = 0.99$). However, when decanoylsucrose was used in the formulations, the relationship was not entirely linear, rather a sharp increase in $AUC_{0\rightarrow360}$ was observed when concentration was increased from 0.125% to 0.25% ($P < 0.05$). This discrepancy is probably because of the fact that 0.125% is below the CMC of decanoylsucrose (0.124%), while 0.25% is above the CMC. The efficacy of the agents also increases with the increase in hydrophobic chain length. The data presented in Figure 3 demonstrates that enoxaparin absorption was enhanced in a chain-length- and dose-dependent manner for all alkanoylsucroses and concentration tested. The chain-length dependency of the effect of alkanoylsucroses is consistent with the effect of alkylglycosides on nasal absorption of insulin. Earlier reports (Pillion et al 1994, 2002; Ahsan et al 2003a) have demonstrated a structure–function relationship among various biochemically synthesized alkylglycosides, including two acyl esters of sucrose, sucrose monodecanoate and sucrose dodecanoate. Insulin permeation was minimally enhanced in the presence of purified alkylglycosides with a shorter alkyl chain linked to glucose, whereas alkylglycosides with a longer hydrophobic chain attached to a maltose ring caused maximum increase in insulin absorption. Since both alkylmaltosides and alkanoylsucroses enhance nasal absorption of insulin and enoxaparin, two structurally different drug molecules, it can be argued that these agents act directly on nasal membrane rather than interacting with the drug molecule. Further, in a recent study it has been shown that tetradecylmaltoside increases insulin transport across 16HBE14o$^-$ via both paracellular and transcellular pathway (Ahsan et al 2003b). Tetradecylmaltoside also causes a decrease in the transepithelial resistance of the cell monolayers and causes loosening of cell–cell tight junctions. Since alkanoylsucroses share the same chemical features as of that tetradecylmaltosides, it can be argued that these agents also act by the similar mechanism.

Chain-length- and concentration-dependent effects of alkanoylsucroses can perhaps be best explained by the CMC of the agents. As they enhance absorption by a direct effect on nasal epithelium, it is reasonable to assume that an increase in the number of micelles will increase the absorption of a drug. In fact, it is well documented that micelles can solubilize the lipid skeleton of biomembranes and facilitate the transport of drug across epithelial membranes (Lichtenberg et al 1983). When a surfactant has a long carbon chain, it is expected to increase micelle size with the square of the alkyl chain length. It has also been reported that the number of micelles increases with the number of carbons in the alkyl chain of a surfactant (Yalkowsky 1996). Increasing the chain length of the surfactant increases the solubilization capacity of the surfactant, thereby producing more drug absorption. These aspects of surfactant micellization are entirely consistent with the finding that dodecanoylsucrose was the most efficacious absorption promoter for nasal enoxaparin, while octanoylsucrose was the least efficacious among the alkanoylsucroses tested. Table 1 shows a reciprocal relationship between the absorption-enhancing effect of alkanoylsucroses and their CMCs. When the CMC of alkanoylsucroses was increased from 0.015% to 1.14%, the nasal enoxaparin absorption decreased in a dose-dependent manner. A similar relationship between alkyl chain length of acylcarnitine and micelles was observed when acylcarnitines were used as absorption enhancers of nasal azetirelin (Kagatani et al 1998). In addition, Jones (1999) pointed out that as the concentration of a surfactant is increased, the initial interaction is between the surfactant monomer and the lipid bilayer. The monomer penetrates into the bilayer until saturation occurs, after which mixed surfactant–lipid micelles can form. Generally, the more hydrophobic the surfactant and the lower its CMC, the greater will be its tendency to penetrate into lipid bilayers at low concentrations. Altogether, the CMC of alkanoylsucroses plays an important role in enhancing their efficacy as nasal absorption promoters.

Reversibility studies

Recently, reversibility studies, both in-vivo and in-vitro, have been proposed as useful methods to study acute effects of permeability enhancers on epithelial membranes or cell monolayers (Vermehren et al 1996; Bagger et al 2001; Arnold et al 2002). For in-vivo reversibility studies, absorption promoters and drugs are administered separately, unlike in-vivo absorption studies for which drugs and promoters are administered together as a single solution. Like in-vivo absorption studies, the effects of the promoters on epithelial membranes are determined by measuring pharmacodynamics and pharmacokinetics of the drug in question. Reversibility studies can provide important insights as to the acute toxicity and duration of action of the agents.

In this study, dodecanoylsucrose was chosen at a concentration of 0.5% for the reversibility study because 0.5% dodecanoylsucrose was found to be the most efficacious in enhancing nasal absorption. When enoxaparin ($330\ U\,kg^{-1}$) was instilled immediately (at time 0) after 0.5% dodecanoylsucrose administration, the enoxaparin absorption showed a trend similar to that obtained from nasal absorption studies. When enoxaparin was administered 1 h after 0.5% dodecanoylsucrose was applied, there was a moderate decrease in C_{max} and $AUC_{0\rightarrow120}$. However, when enoxaparin was delivered 2 h after dodecanoylsucrose application, the $AUC_{0\rightarrow120}$ ($34.7 \pm 2.3\ U\,min\,mL^{-1}$) and C_{max} ($0.38 \pm 0.01\ U\,mL^{-1}$) decreased significantly (Figure 4A). It is worthwhile to note that there was a 1.5-fold decrease in the $AUC_{0\rightarrow120}$ when enoxaparin was administered 2 h after 0.5% dodecanoylsucrose administration (Figures 4 and 5). $AUC_{0\rightarrow120}$ values presented in Figure 5 show that the effect on dodecanoylsucrose

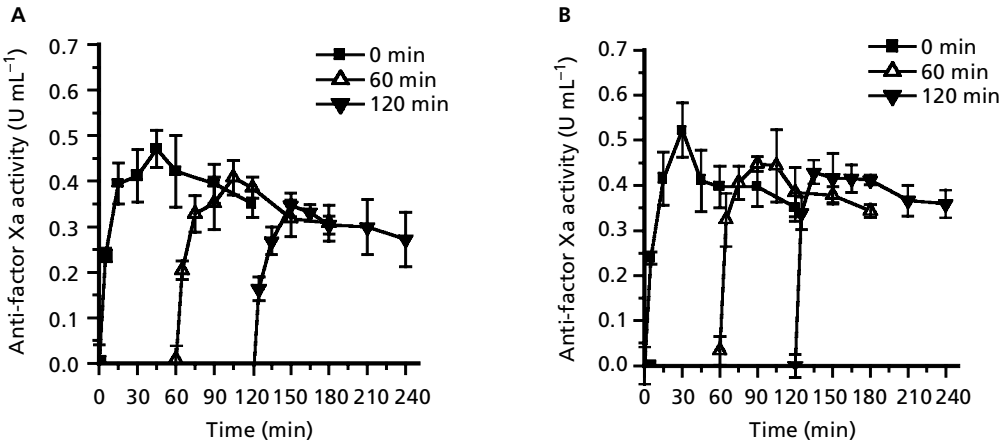


Figure 4 Changes in plasma anti-factor Xa activity following nasal administration of 330 IU kg^{-1} enoxaparin to anaesthetized rats at time 0, 60 or 120 min post administration of 0.5% dodecanoylsucrose (A) or 1% sodium glycocholate (B). Data represent mean ± s.e.m., n = 3–5.

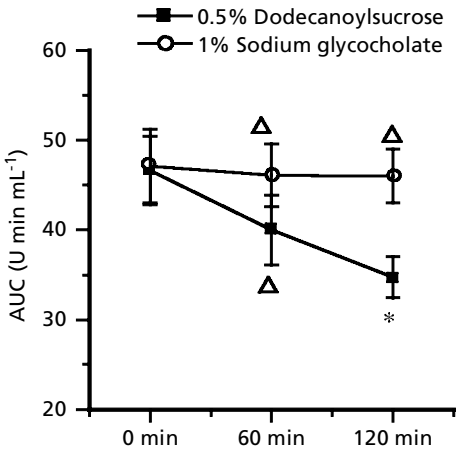


Figure 5 Time-dependent effect of dodecanoylsucrose and sodium glycocholate on nasal absorption of enoxaparin observed during reversibility study in anaesthetized rats. Data represent mean ± s.e.m., n = 3–5. $^{\triangle}P > 0.05$ (not significant) compared with administration of enoxaparin at time zero after the enhancer administration; $^{*}P < 0.05$ (significant) compared with the administration of enoxaparin at time zero after the enhancer administration.

diminishes with time. Consequently, it can be argued that the increased permeability of the nasal epithelium caused by acute exposure to 0.5% dodecanoylsucrose is reversible, suggesting that short-term exposure to alkanoylsucroses is less likely to cause an extensive damage or cellular toxicity in the nasal epithelial cells. A parallel series of reversibility experiments were also conducted utilizing 1% sodium glycocholate in nasal enoxaparin formulations (Figure 4B). The effect of sodium glycocholate does not appear to diminish with time (Figures 4B and 5). There were no appreciable changes in C_{max} or $AUC_{0\rightarrow120}$ when enoxaparin was administered 2 h after sodium glycocholate administration compared with enoxaparin administered immediately after sodium glycocholate administration. The effect of sodium glycocholate on the nasal mucosa was maintained throughout the 2-h experiment. In fact, it was reported by Bagger et al (2001) that the enhancing effect of sodium glycocholate was reversible in approximately 4 h. Comparing the data from the reversibility study using dodecanoylsucrose with that of the study using sodium glycocholate, it can be concluded that alkanoylsucroses are less likely to cause irreversible damage to the nasal mucosa.

Conclusions

In summary, alkanoylsucroses could be used as absorption promoters for nasally administered LMWHs. Alkanoylsucroses enhance nasal drug absorption in a dose- and chain-length-dependent manner, with dodecanoylsucrose being a more efficacious absorption promoter than decanoylsucrose or octanoylsucrose. Dodecanoylsucrose is twice as potent as sodium glycocholate, a widely used nasal absorption promoter. The effect of dodecanoylsucrose on nasal epithelium disappears in 2 h, while that of sodium glycocholate continues for more than 2 h. However, more chronic histological studies need to be done to determine the long-term effects of alkanoylsucrose on the integrity of the nasal epithelial membrane.

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